Proposed Perchlorate Studies

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Introduction

Perchlorate has recently been recognized as a persistent and pervasive contaminant of water supplies in a number of major metropolitan areas. Current efforts at assessing the health risks of perchlorate have been hampered, not by lack of understanding of the mechanism of action, but instead by a lack of relevant data (Terra, 1997). Perchlorate is known to disrupt thyroid hormone homeostasis in a number of species via an inhibition of iodine uptake into the thyroid gland (e.g., Mannisot et al., 1979; Wyngaarden et al., 1952; Stansbury and Wyngaarden, 1952; Lampe et al., 1967). What is still currently unknown is whether there are differences in the sensitivity to perchlorate of the developing organism versus the mature adult organism. Also unknown is whether there are significant differences in the sensitivity of the Iodine-transporter in the thyroids of different species.

Ongoing studies on the health effects of perchlorate include an 90-day subchronic drinking water study and a developmental neurotoxicology study. These studies should provide data on some of the potential health effects of perchlorate. However, there will still be uncertainties in the risk assessment process, including an uncertainty in the extrapolation from animal data to humans, and an uncertainty due to potential age-dependent differences in the toxicity of perchlorate.

This proposal aims to collect data that will increase the biological basis that underlies these two uncertainties. First, careful characterization of the effects of perchlorate on thyroid hormone homeostasis in the developing rodent will allow a for a clear age comparison with the data from the 90-day subchronic study. Second, data from ex vivo and/or in vitro studies will allow a direct comparison of the sensitivity of the iodine-transporter to perchlorate in humans and rodents. These data will be significant in deciding the degree to which the above mentioned uncertainty factors are necessary in extrapolating data from animal studies to humans.

Phase One: Characterization of the effects of perchlorate in the developing animal.

Background: It is clear that perchlorate inhibits iodine uptake and results in hypothyroidism in a number of species (e.g., Mannisot et al., 1979; Wyngaarden et al., 1952; Stansbury and Wyngaarden, 1952; Lampe et al., 1967). What remains to be determined is whether the developing animal is more, or less, sensitive than the adult. Further research should include a developmental study with gestational and postnatal time points for both maternal and fetal/pup thyroid hormones (THs) and TSH. Due to the critical role of thyroid hormones in the development of the nervous system (see Stein et al., 1991; Porterfield, 1994) it is prudent to measure biomarkers of nervous system structure and/or function in perchlorate exposed offspring. Although Argus will conduct a developmental neurotoxicity study per EPA guidelines, it will not collect adequate data on the effects of perchlorate on THs in the fetus and pups. A recent review of published literature suggests that circulating concentrations of THs are the sensitive and critical endpoints in developmental neurotoxicity studies of xenobiotics that disrupt thyroid hormone homeostasis (Crofton, 1997).

Proposed Work:
Initial studies should use exposure conditions that bracket those used in the Argus study (York, 1997). This includes time of exposure and concentrations. However, the fact that the Argus study cannot continue dosing until weaning (postnatal age 21) is a limitation. Additional work should expand dosing until weaning. Endpoints to be assessed in the initial studies should include: T4, T3 and TSH and auditory function.

1) THs: Time points for maternal sampling should include: gestational days (GD) 10* and 21, and postnatal days (PND) 3, 14, 21. Some flexibility will be needed to match the Argus study since it ended dosing on PND10 (Need to obtain maternal samples at this time point). Fetal and pup time points should include: GD10 and 21, and PND 3, 7, 14, 21, 35 and ✇adult■. The first time point (GD10) is needed because this is the time during which maternal THs are crucial for fetal development (Porterfield, 1994). The fetal thyroid is ✇inactive■ at this point and the developing CNS is entirely dependent on maternally derived thyroid hormones (TH). Maternal exposure to perchlorate is known to adversely impact the fetal thyroid (Postel, 1957; Sztanyik and Turai, 1988). The rationale for the later fetal time point GD21, is that it gives us an indication of whether or not the fetus is hypothyroid at a time when the fetal thyroid is actively sequestering iodine and excreting THs. Maternal levels at this time do not necessarily translate to fetal levels. The postnatal time points are those that have been successfully used in the past to monitor the ontogeny of postnatal thyroid hormones in the rat (Goldey et al., 1995a; 1995b).

* GD10 time point - really any time from GD10-GD15 or 16 could be used. It may be problematic to get fetal TH measurements at the earlier time points due to small serum volumes (pooled fetuses from pooled litters may be needed).

2) Auditory system function: The auditory system of the rat is extremely vulnerable to alterations in thyroid hormone status during the early postnatal period, a time of ongoing cochlear development (Uziel et al., 1980; 1981). Auditory thresholds are easily measured (Crofton, 1992) and provide a clear nervous system biomarker for developmental hypothyroidism (Goldey et al,1995a;Herr et al.,1996; Meza et al., 1996). This procedure is well validated and has been used for over two decades in animal toxicology and human clinical studies (see Crofton, 1992 for review).

Value of Results: The results of this first phase should provide adequate data for a comparison of fetal/pup THs to the adult data from the 90-day study. If the adult is found to be more sensitive, then no further work is needed in the developing organism.

Resource Needs: This first will require a 12-18 month time frame with funding of a post-doc or tech slot. Approximate costs: personnel = $50-75k, supplies and equipment = $5-10k (mostly for RIA kits).

Phase 2: Data to support extrapolation from animal data to humans.

Background: Current data from animal models suggests that, at least for thyroid tumors, rodents are more sensitive to the TSH feedback mechanism that triggers hyperplasia and subsequent formation of thyroid neoplasia (Capen, 1994; 1997; McClain, 1995). This is a difference in the proliferative response of the thyroid to long term TSH stimulation, and not necessarily due to the pharmacological effect of the xenobiotics on the thyroid gland. Perchlorate works through inhibition of iodide uptake and I am unaware of data that will allow direct comparison of the sensitivity to perchlorate of the human and rodent thyroid glands. Simple in vitro thyroid uptake experiments should determine whether there is any difference in susceptibility of the developing and mature thyroid gland.

Proposed Work:

1) In vitro iodine uptake: Tissue slices from fetal, postnatal and adult thyroid gland can be incubated with ^{125}I (see Gaitan et al, 1983). This will provide comparative dose response data for the sensitivity to perchlorate of different age thyroid glands. This same type of experiment can be done with animal vs. human tissue (rate limiting factor will be obtaining human thyroid tissue at appropriate ages). Similar experiments could be done to examine the effects of perchlorate in vitro against cultured cells from the two species (e.g., Atterwill and Fowler, 1990).

2) In vivo iodine uptake: Rats fed low iodine diets are injected with perchlorate followed by ^{125}I. Thyroid gland ^{125}I concentrations are measured (see Gaitan and Cooksey, 1989). These experiments in rats will provide in vivo data to compare to the in vitro studies above.

Value of Results: These data should provide the necessary information to determine

whether there is a species difference in the effects of perchlorate on inhibition of iodine uptake. These data will be useful in determining the necessity or extent to which an uncertainty factor should be used in the RfD process for perchlorate.

Resource Needs: This phase will require a 12 month time frame with funding of a post-doc or tech slot. Approximate costs: personnel = $50k, supplies and equipment = $5-10k (need radiolabeled iodine, tissue incubator and other minor lab equipment). It is possible, with adequate resources that this phase could be run concurrently with Phase 1.

Phase 3: Role of Hypothyroidism in the Effects of Perchlorate and Possible Biomarkers of Perchlorate Neurotoxicity:

Background: The complexity and plasticity of the developing nervous system make it difficult at times to associate the effects of xenobiotics with specific mechanisms. This is especially true when the endpoints are functional evaluations. It is possible that any developmental neurotoxicity (e.g., hearing loss, any functional effects seen in the Argus study, or see below for biochemical markers) induced by maternal perchlorate exposure may be due to other than a thyroid-mediated mechanism.

Another potential problem with the use of functional evaluations is that they may, or may not, be sensitive biomarkers of exposure. The tests in the EPA guidelines were designed as ✇screens■ for use with chemicals for which no mechanistic data is available. These tests were not specifically designed to monitor xenobiotic-induced disruption of thyroid hormones during development. In addition, there was no requirement in the Argus contract to provide data demonstrating the sensitivity of their assays to developmental hypothyroidism. These facts, coupled with the known plasticity of the nervous system during development (ref), and the general failure of the behavioral models to detect anything other than massive damage to the cerebellum (a known target of thyroid hormones during development) point to a clear need for other biomarkers of neural development in perchlorated exposed animals.

Proposed Work:

1) Thyroid hormone replacement experiments: These studies would be used to determine the role of hypothyroidism in any neurotoxicity detected following developmental exposure to perchlorate. These studies would involve daily injection of T3 and T4 to the dam and/or offspring (see Escobar-Morreale et al., 1995), measurement of THs, and appropriate functional/biochemical endpoints. This TH therapy should alleviate the effects, if said effects are due to alterations in thyroid hormones (see for example Goldey and Crofton, 1997). A lack of therapeutic effect of thyroid hormones would suggest that there are non-thyroidal effects of perchlorate. Note that these experiments will only be needed if perchlorate effects on the nervous system are seen at or below the LOEL effects for THs.

2) Biochemical/anatomical markers of perchlorate-induced developmental

neurotoxicity: I would also like to include some biomarkers such as myelin basic protein or Pcp-2 (e.g., northern, RT-PCR or western blots) in the cerebellum (e.g., Zou et al., 1994; Farsetti et al., 1992; Martinez-Galan et al., 1997), or some of the CNS proteins that Tom Zoeller (Univ. Mass., personnel communications) has identified as being linked to T3-response elements in fetal brain tissue.

Value of Work: These studies would provide data to clearly distinguish thyroid-mediated mechanisms from other effects of perchlorate. Additionally, these experiments are important to determine the sensitivity of various biomarkers in the developing CNS. I think that circulating concentrations of THs will be the most sensitive indicators of developmental neurotoxicity in studies of xenobiotics that disrupt thyroid hormone homeostasis (Crofton, 1997). This work, along with the auditory function data collected in Phase I above and the data from the Argus study, will support or refute this hypothesis.

Resource Needs: Addition of this work would make the entire project into a 3 year effort and require the funding of a post-doc for 3 years ($150k) and about $10k per year for supplies.

Summary

In summary, this research will provide data that will decrease the uncertainties in the health risk assessment for perchlorate. Careful characterization of the potential age-dependent thyrotoxicity of perchlorate will address the potential problem of fetal and neonatal ages as sensitive sub-populations. Data from ex vivo and/or in vitro studies will allow a direct comparison of the sensitivity of the iodine-transporter to perchlorate in humans and rodents, thus providing a biological basis for the animal-to-human uncertainty factor at the mechanistic level. In addition, these proposed experiments will allow a differentiation of potential thyroid-mediated developmental neurotoxicity from non-thyroid mediated developmental neurotoxicity. Overall, these data will be important in deciding the degree to which the above mentioned uncertainty factors are necessary in extrapolating data from animal studies to humans.

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